Growth Factor and Cytogenetic Abnormalities in Cultured Nevi and Malignant Melanomas*

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It has been proposed that benign nevi that fail to differentiate normally may undergo stepwise growth and morphologic changes resulting in progression toward dysplastic nevi, which in some cases progress into malignant melanoma. In this study, we sought to determine the relationship between production of endogenous growth factors and the appearance of chromosomal abnormalities in cultured nevi and melanomas. Newly established cultures from 8 nevi with benign histology and 6 malignant melanomas, and 2 malignant melanoma cell lines were studied. Assays for mitogenic growth factors were based on stimulation of [^{3}H]thymidine incorporation into DNA in Hs0294 malignant melanoma cells, produced by serum-free conditioned medium from nevus or melanoma cultures. Karyotypes were examined in cultures of an equivalent passage. Three of the 8 nevus cultures were mitogen-negative and displayed normal karyotypes; one nevus culture was mitogen-positive and had a normal karyotype, although the biopsied tissue demonstrated histologic evidence of benign melanocytic proliferation; one was mitogen-negative initially, but had an extra chromosome 8 in 2 of 50 cells; 3 were mitogen-positive and chromosomally abnormal. Each of the cultures in this latter group exhibited reciprocal translocation (rcpt) as the only identifiable abnormality [rcpt(6;15), rcpt(10;15), rcpt(15;20)], or a constitutional rcpt(4;5). Thus, there was direct correlation between growth factor production and chromosome abnormality in 6 of 8 benign nevus cultures.

In the newly established melanoma cultures there was also concordance between growth factor and chromosomal status; conditioned media from 4 of 6 were mitogen-positive by at least one assay, and all 4 of the mitogen-positive cultures had chromosomally abnormal cell populations. Of the 2 melanoma cultures negative for growth factors, one was also negative for chromosome abnormality; the other had chromosomal change consisting of increased polyploidy. Both melanoma cell lines had abnormal karyotypes and were mitogen-positive. Though numerous chromosome changes were noted in the karyotypically abnormal melanoma cells, 6 of the 8 cultures exhibited abnormalities in chromosomes 1, 6, and/or 7.

These data suggest that steps in the progression from benign nevi toward dysplastic nevi or malignant melanoma include: (1) proliferation resulting from altered production of endogenous mitogenic growth factors; and (2) development of specific chromosomal abnormalities. *J Invest Dermatol 86:295–302, 1986*

The sequence of events involved in the progression of a normal melanocyte to a malignant melanoma has been described by Clark et al [1] in 5 steps: (1) proliferation of benign cells in small foci; (2) development of random atypia with failure to differentiate, resulting in an abnormal pattern of cell growth; (3) autonomous growth, often associated with invasion; (4) formation of a primary cancerous lesion with competence for metastasis; (5) metastasis. These investigators are thus implying that alteration of cell growth is an initial step toward malignancy. Early proliferation of melanocytes may result from altered production of or response to an autostimulatory growth factor [2]. Chromosome abnormalities may precede, accompany, or follow altered cell growth as the nevus progresses to a melanoma. Our laboratory has demonstrated that melanoma cells produce an autostimulatory monolayer mitogen, termed MGSA for melanoma growth stimulatory activity [3]. In the studies reported here we sought to assess the prevalence of production of melanoma mitogens in human malignant melanoma cultures and in newly established cultures of benign nevi, and to determine whether chromosomal aberrations or histologic abnormalities could be linked to release of these mitogens.

Manuscript received April 23, 1985; accepted for publication September 23, 1985.

This study was supported by Veterans Administration Innovative Cancer Research Grant and National Cancer Institute Grant 1 R23 Ca 34590. Dr. Lawson is the recipient of a V.A. Career Development Award.

*Presented in part to The Society for Investigative Dermatology, Washington D.C., May 1985.

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Abbreviations:
DMEM: Dulbecco's modified Eagle's medium
F-10: Ham's F-10 medium
FBS: fetal bovine serum
NRK: normal rat kidney
PBS: phosphate-buffered saline
rcpt: reciprocal translocation
TGF: transforming growth factor

MATERIALS AND METHODS

Patients and Cultures This study was approved by the Human Investigations Committee at Emory University. Primary cultures were established from tissue samples obtained after obtaining informed consent from melanoma patients undergoing surgical resection or from dermatology patients having nevi biopsied for histologic examination. The melanoma cell line Hs0294 was obtained from Naval Biological Supply, Oakland, California. The

Table IA. Clinical Features of Nevus Patients

Patient (Nevus Number)	Site of Lesion	Skin Type[a]	Ancestry	Family History: Melanoma	Family History: Excessive Nevi[b]	Family History: Cancer	Histology
N-1	Trunk	2	Scottish-Irish and French	No	Yes	No	Compound melanocytic nevus
N-2	Trunk	2	Irish	Yes	No	Yes	Compound melanocytic nevus
N-3	Extremity	1	English	No	No	No	Compound melanocytic nevus
N-4	Trunk	1	Scottish-Irish	No	No	No	Compound melanocytic nevus
N-5, N-6	Trunk	2	German and English	Yes	Yes	Yes	Intradermal melanocytic nevus
N-7	Extremity	2	English	No	Yes	Yes	Compound melanocytic nevus
N-8	Trunk	1	Scottish and German	Yes	Yes	Yes	Compound melanocytic nevus

[a]Skin typing was according to Fitzpatrick [4].
[b]These data are based upon patient impressions as to whether other members of their family had a greater than average number of nevi.

Ca-Gor melanoma cell line was established and donated by Dr. William Cassel, Emory University. All of these tissues obtained were prepared for histologic examination. With 2 exceptions, all the nevi that grew well enough in cell culture to be studied were classified as compound melanocytic nevi (Table IA). All the melanoma patients had recurrent disease (Table IB), and the stage assigned is based on nodal (N) and metastasis (M) status [5]. None of the melanoma patients had received either radiation therapy or chemotherapy, which possibly could have induced chromosomal abnormalities, before these lesions were removed. Two of the six patients (M-1 and M-3) had received injections of a viral oncolysate as adjuvant therapy after their initial resections [6].

Culture Conditions and Chromosome Preparation After scissor excision the nevus specimens were incubated in a trypsin/EDTA solution (0.05%/0.025%) at 4°C overnight. The next day the dermis and epidermis were separated and tissue was mechanically disaggregated as previously described [7]. The resulting cell suspension was cultured in MCDB-151 medium (GIBCO) supplemented with 20% fetal bovine serum (FBS), gentamicin (50 μg/ml), and fungizone (25 μg/ml). Melanin-producing cells were identified by microscopic examination of cells after the L-dopa-tyrosinase assay [8].

Melanoma tissue was mechanically disaggregated according to procedures of Meyskens et al [9]; in some instances the tissue was also subjected to collagenase or trypsin digestion. Cells were maintained in Ham's F-10 medium (F-10) supplemented with 20% FBS, gentamicin (50 μg/ml), and fungizone (25 μg/ml). After every 3 passages cells from newly established cultures were frozen for future studies. Melanoma cultures were evaluated for melanin production by the same procedure used for nevus cultures.

For chromosome studies, cultures established from nevi or melanomas were treated with colcemid (final concentration 0.025–0.05 μg/ml) for 60–90 min; cells were suspended from monolayer with trypsin (0.25%; GIBCO), and treated with a hypotonic KCl solution (0.075 M at 37°C for up to 30 min). After fixation with absolute methanol:glacial acetic acid (3:1), air-dried slides were prepared for G banding using trypsin and Giemsa stain banding [10]. Cytogenetic characterizations were based on an average of 30 (range 12–50) eye karyotypes for study of nevi and an average of 22 (range 5–49) eye karyotypes of melanomas. There were at least 2 banded photograph karyotypes per study (range 2–17). Tetraploidy was assessed by the ratio of 4n/2n + 4n metaphases expressed as a percent. At least 100 metaphases were examined for each analysis of tetraploidy.

Melanoma Mitogen Assay Confluent cultures of nevi or melanomas were washed twice with phosphate-buffered saline (PBS) and placed in serum-free medium. After 48 h the medium was replaced with fresh serum-free medium. Subsequently, every 48 h the medium was changed. Culture medium conditioned by the nevus or melanoma culture was collected, centrifuged at 1500 *g* to remove any cells, and the supernate stored frozen at −80°C. Three collections were made from each culture, allowing a maximum time in serum-free medium of 8 days, collections being taken only on the last 6 days. Mitogenic activity was assayed as previously described [3,7]. Briefly, low-density cultures (6000

Table IB. Clinical Features of Melanoma Patients

Patient Number	Age/Race/Sex	Site of Primary Lesion	Lesion from Which Culture was Established	Stage	Time of Recurrence (Months)[a]
M-1	56/W/M	a. Mid back b. Left wrist	Left cervical node	IV	23
M-2	66/B/M	Plantar surface of right foot	Right femoral node	III	22
M-3	23/W/F	Right tibial surface	Iliac and obturator nodes	IV	66
M-4	48/W/F	Right thigh	Intra-abdominal lymph node and viscera	IV	10
M-5	66/W/M	Right ear	Right cervical node	III	14
M-6	74/W/F	Right heel	Pelvic and inguinal nodes	IV	18

[a]Time from primary resection to this resection.

cells per scintillation vial) of Hs0294 melanoma cells were depleted of serum for 24 h, washed twice with PBS, and placed in serum-free F-10 medium. The next day, culture medium was aspirated and a 1:10 dilution of serum-free conditioned medium from nevus or melanoma cultures was added to each of 3 vials. Six hours later 5 μCi of [^{3}H]thymidine (26.5 Ci/mmol) were added to each vial and cultures were pulsed for 18 h. At that time the radioactive medium was decanted and the cells were fixed with methanol:ethanol (3:1). Free [^{3}H]thymidine was removed by subsequent washes with absolute methanol. Fixed cells were solubilized in the vials with scintillation fluid and radioactivity was counted on a Beckman LS 1800. A positive mitogen value was assigned to 1:10 dilutions of medium which stimulate [^{3}H]thymidine incorporation at least 35% more than does the nonconditioned serum-free medium in control vials. Both mitogen and chromosome assays were performed on primary cultures or at the earliest possible passage number (at a 1:2 split) after primary culture.

Transforming Growth Factor (TGF) Assays The procedures of Todaro et al [2] were used to determine whether TGFs were being produced by the melanoma cells in culture. Briefly, the cells to be evaluated for TGF production were placed in a soft agar feeder layer [Dulbecco's modified Eagle's medium (DMEM), 10% calf serum, 0.5% Noble agar] at a density of 5×10^4 cells per 35-mm culture dish. The overlayer (DMEM, 10% calf serum, 0.3% Noble agar) contained normal rat kidney (NRK) indicator cells (clone 49F) which respond to TGFs α and β by forming colonies in soft agar. The indicator cell density was 2000 cells/35-mm culture dish. Cultures were placed in a water-jacketed incubator at 37°C with 95% air/5% CO_2. Every 7 days the cultures were fed with 1 ml of DMEM containing 10% calf serum and 0.5% agar. After 21 days the number of NRK colonies in each culture dish was determined by manual count using a Unitron inverted phase microscope. A colony is defined as a group of more than 10 cells. The total amount of TGF-α activity is proportional to the number and size of soft agar colonies formed. Transforming growth factor-β production was assessed in a similar manner, except that epidermal growth factor (2 ng/ml) was included in the culture medium of the overlayer with the NRK cells [11]. Cultures were defined as positive for TGF assays when the total number of NRK colonies formed in response to the feeder layer was at least 25% greater than the controls without feeder layer.

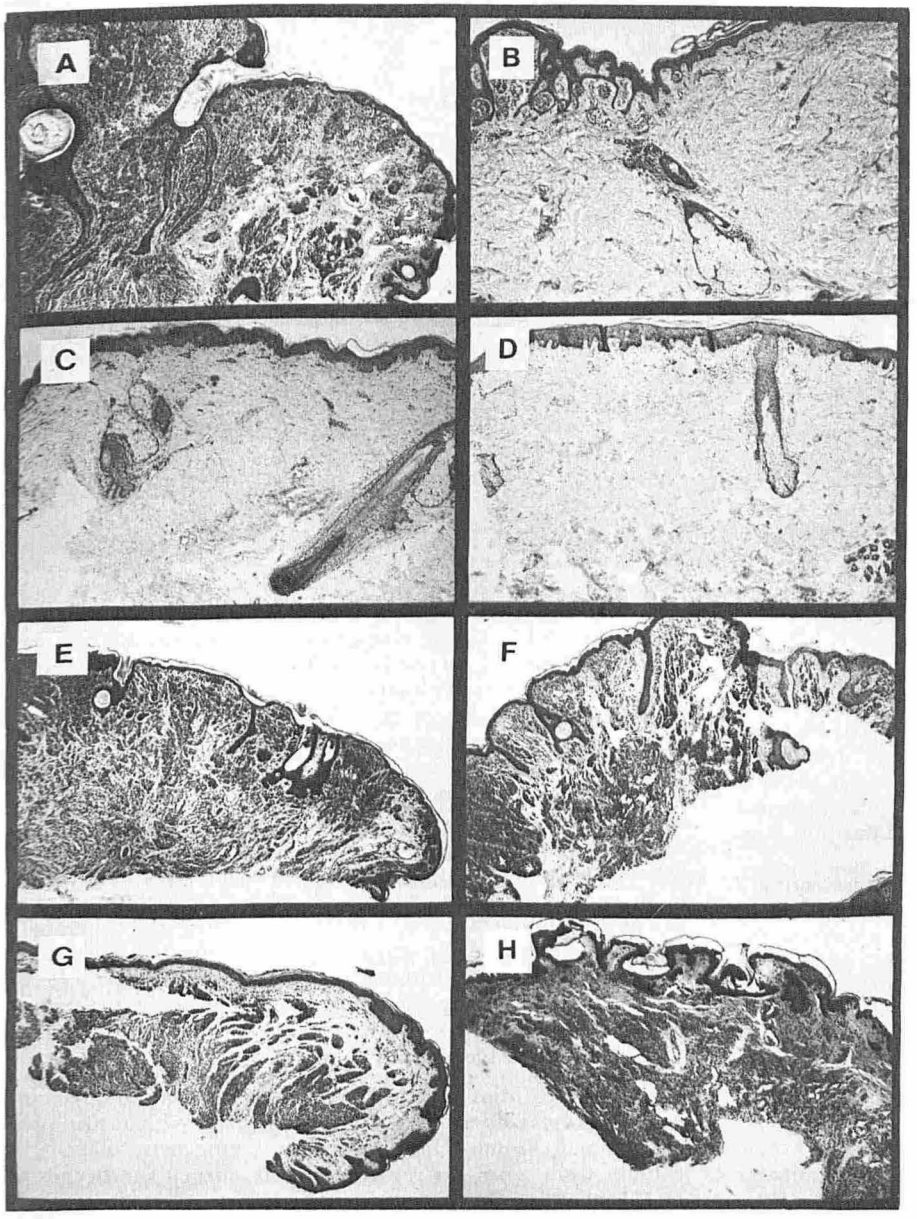

Figure 1. Photomicrographs showing "shoulders" of nevi. N-1 through N-8 (*A–H*, respectively) show no significant melanocytic proliferation at the shoulder (periphery) of the nevi. In N-2 (*B*) a solitary compact theque and individual melanocytes are apparent in the basal cell zone, but no atypia is apparent.

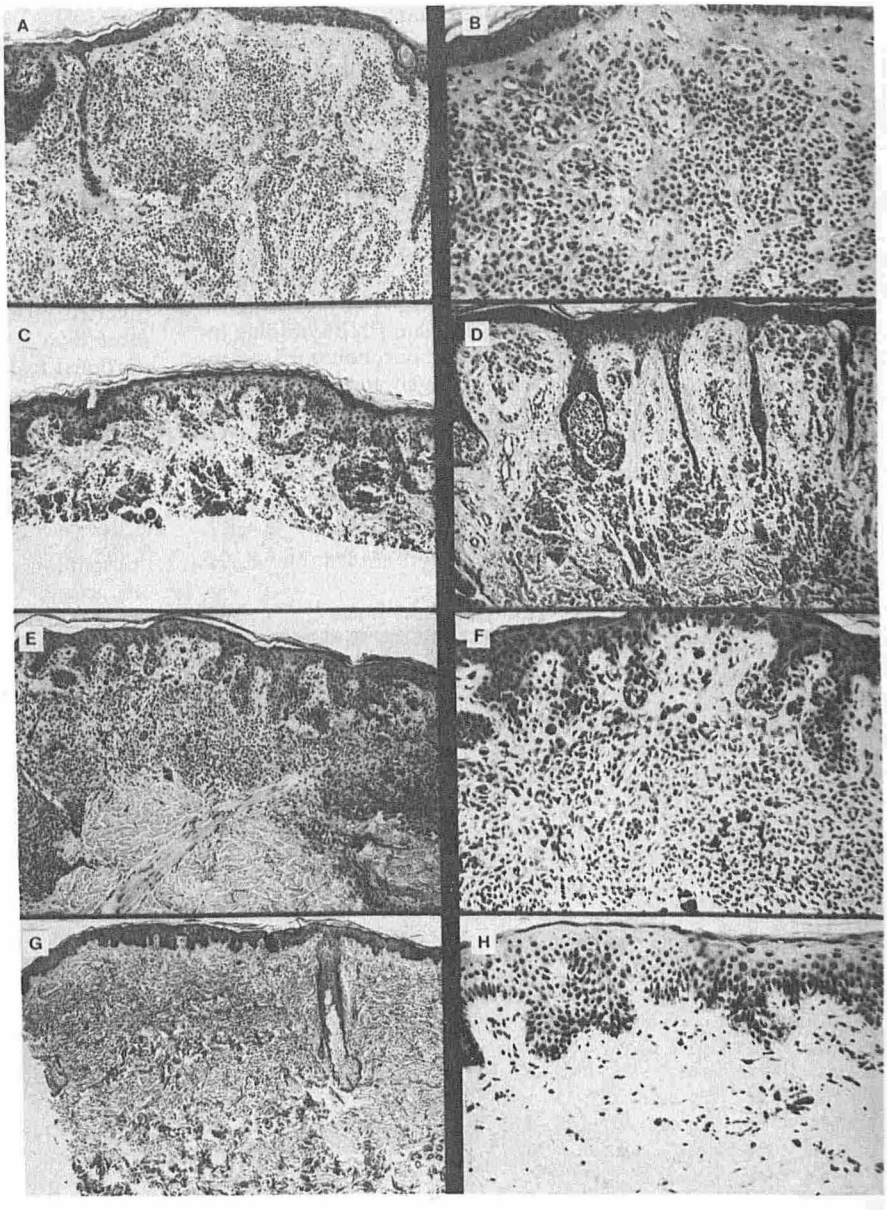

Figure 2. Photomicroscopy of histologic sections from nevi 1–4. *A*, (N-1) Normal-appearing type A and B melanocytic nevus cells fill the upper $\frac{1}{5}$ of the reticular dermis. Prominent individual melanocytes are present in the basal cell layer. H&E, × 4.5. *B*, (N-1) Theques of uniform intradermal melanocytic nevus cells with light dusty pigment are present in the upper reticular dermis surrounded by islands of type B melanocytic nevus cells. H&E, × 10. *C*, (N-2) Compound melanocytic nevus with theques of intraepidermal nevus cells along the dermal-epidermal junction. Benign-appearing type B intradermal nevus cells in the upper reticular dermis. H&E, × 4.5. *D*, (N-2) Compound melanocytic nevus with intraepidermal nevus cells in well-formed compact theques along the dermal-epidermal junction. Benign-appearing type A and B intradermal nevus cells in the papillary and upper reticular dermis. Minimal perivascular round cell infiltrate and edema in the expanded papillary dermis. H&E, × 10. *E*, (N-3) Intraepidermal proliferation of melanocytic nevus cells individually and in theques irregularly distributed along the basal cell layer associated with a normal-appearing component of type B melanocytic nevus cells in the upper $\frac{2}{3}$ of the reticular dermis. H&E, × 10. *F*, (N-3) Epidermal theques and intradermal melanocytic nevus cells. No atypia noted. H&E, × 20. *G*, (N-4) Irregular acanthosis with minimal theque formation and prominent individual melanocytes along the basal layer. There is a sparse mononuclear infiltrate dispersed throughout the upper portion of the reticular dermis. H&E, × 4.5. *H*, (N-4) Focal proliferation of normal-appearing intraepidermal nevus cells individually and in theques along the basal cell layer. No atypia noted. H&E, × 20.

RESULTS

Nevus Studies—Histology and Cytogenetics Since the stage of differentiation for nevi is variable, it was important that each be evaluated histologically prior to culture (Figs 1–3). None of the 8 studied was classified by the pathologist as "dysplastic" (Table IA). However, histologic study revealed that 4 of the nevi (N-1, N-2, N-3, N-4, Table IA) had areas of intraepidermal melanocytic proliferation at the dermal-epidermal junction (Fig. 2, A–H).

Cytogenetic analysis of the 8 nevus cultures (representing 7 patients) revealed entirely normal karyotypes in 4 of 8 (Table II, N-2, N-3, N-6, N-7). Of the 4 with abnormal karyotypes (Fig 4), one had a constitutional abnormality, a rcpt(4;5) in 100% of the cells (Table II, N-8). The remaining 3 abnormals had a mixture of normal and abnormal cells; one had a rcpt(6;15) in 14 of 50 cells and a rcpt(15;20) in 2 of 50 cells (Table II, N-5); one had a rcpt(10;15) in 79% of the cells (Table II, N-1); and the last had an extra chromosome 8 in 2 of 50 cells (Table II, N-4).

Nevus Patients—Monolayer Mitogen Production Three of the nevus cultures had normal karyotypes and negative mitogen levels (Table II, N-2, N-6, N-7). Conditioned medium from one other nevus culture (N-3) also contained mitogenic activity but the cells had normal karyotype. N-3 was established from tissue which exhibited intraepidermal proliferation of melanocytic nevus cells and may represent the earliest stage in nevus progression, i.e., growth factor activation but no karyotypic abnormalities. Conditioned medium from 3 of the 4 chromosomally abnormal cultures contained mitogenic activity (Table II, N-1, N-5, and N-8).

The clinical features (Table IA) were not obviously different between patients with normal karyotype and negative mitogen levels in nevus cultures and patients with abnormal karyotypes and positive mitogen levels. The following categories previously reported to have etiologic significance were compared: age, ethnic origin, medication, sunbathing frequency, and skin type. Nevus cultures from 2 of the 3 patients with a family history of melanoma had chromosome abnormalities and growth factor production (N-5 and N-8, Tables IA and II).

Melanoma Studies In the newly established melanoma cultures there was direct correlation between growth factor production and chromosome abnormality. Cytogenetic analysis of cultures from 6 patients revealed entirely normal karyotypes in one (Table III, M-1). M-1 cultures were also negative for growth

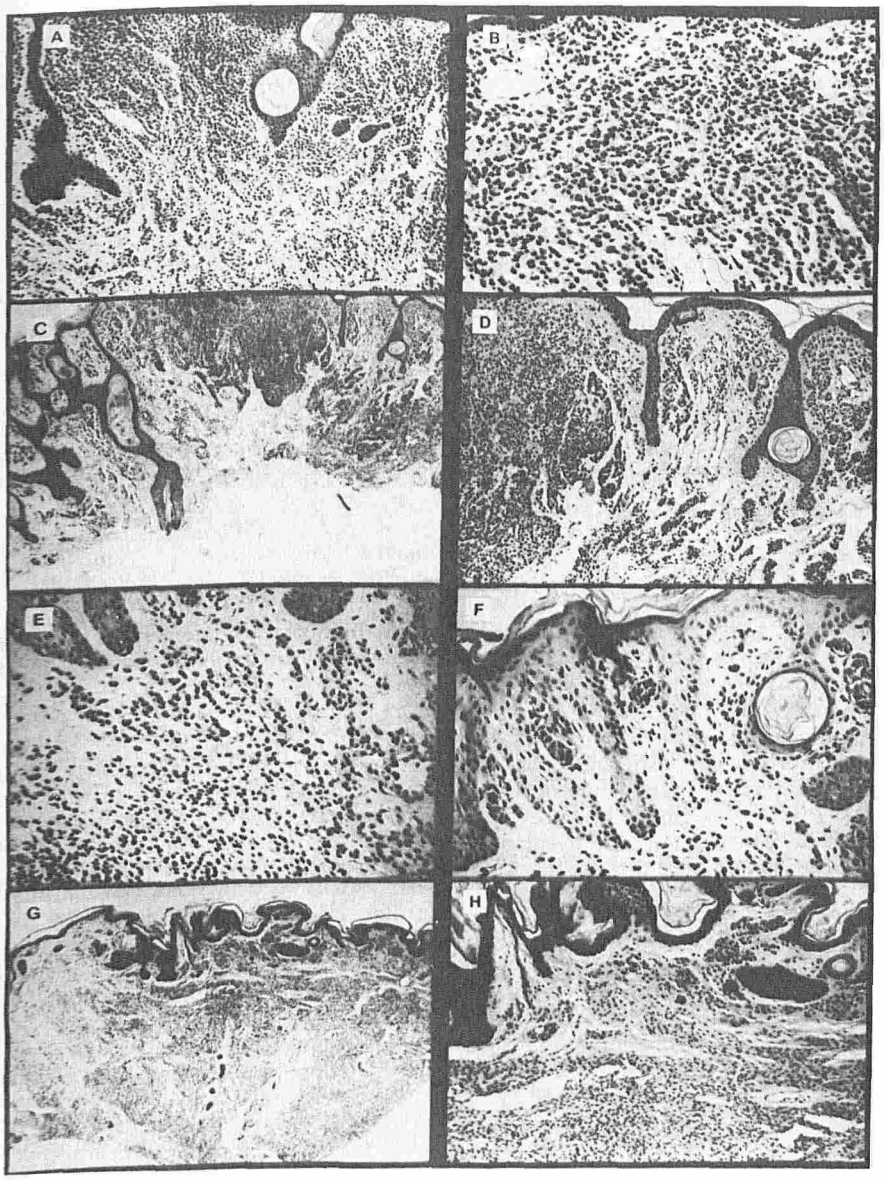

Figure 3. Photomicroscopy of histologic sections from nevi 5-8. *A*, (N-5) Orderly strands and nests of uniform benign-appearing intradermal melanocytic nevus cells in the papillary and reticular dermis. H&E, × 10. *B*, (N-5) Prominent individual melanocytes. No junctional theques are present. H&E, × 20. *C*, (N-6) Prominent elongation of the rete ridges and prominent individual melanocytes are present in the basal cell layer and there are large nests of benign-appearing melanocytic intradermal nevus cells filling the papillary and upper reticular dermis. H&E, × 4.5. *D*, (N-6) Higher power shows a clearer view of intraepidermal melanocytes and intradermal nevus cells. H&E, × 10. *E*, (N-7) Benign-appearing type C neuroid nevus cells present in the reticular dermis. Dilated vascular channels are present. H&E, × 20. *F*, (N-7) Theques of benign-appearing intraepidermal nevus cells containing variable amounts of fine pigment granules at the dermal-epidermal junction and benign type A and B intradermal nevus cells in the papillary and upper reticular dermis. A pseudo horn cyst is present at the base of a rete ridge. H&E, × 20. *G*, (N-8) Scanning power showing a benign intradermal nevus with no significant junctional component. H&E, × 4.5. *H*, (N-8) Higher power showing predominantly type B and C intradermal nevus cells present in the papillary and reticular dermis. Vascular dilatation is prominent. H&E, × 20.

factors and melanin. Melanoma cultures from a second patient (Table III, M-2) had a missing Y chromosome in 2 of 42 cultured cells and the remaining were chromosomally normal. Loss of the Y is not uncommon in cells from older males, unrelated to tumor formation. These M-2 cultures were positive for mitogen activity and also for melanin, but negative for TGF activity. Cultures from 3 other patients (Table III, M-3, M-4, and M-6) had both chromosomally normal and abnormal cells. All 3 sets of cultures were positive for tyrosinase activity and one or both of the growth factor assays. Cultures from 1 additional patient had cells with only abnormal chromosomes (Table III, M-5) and both melanin and TGF assays were positive. It should be noted that the karyotypically normal cells in metastatic melanoma may represent inclusion of some normal cells from the surrounding tissue in the initial culture. Further details of the abnormal karyotypes are as follows.

Patient M-3: Four of 11 cells examined were polyploid, 2 in the 4n range and 2 in the 8n range; the remaining 7 cells had normal diploid chromosomes. One of the 8n cells could be fully karyotyped by photography, and there were 4 unidentified marker chromosomes and 2 1p− chromosomes. Thus, changes in passage 1 cultures from M-3 involved polyploidy with loss of the short arm of chromosome 1 and appearance of other marker chromosomes.

Patient M-4: Twelve of 23 cells had normal diploid chromosomes. The remaining cells studied were hyperdiploid in the range of 69–75. At least 10 rearranged and unidentifiable chromosomes were present. A pattern of change in copy number for particular chromosomes could not be identified. Thus abnormality in passage 1 cultures from M-4 involved hyperdiploidy (polyploidization?) and appearance of many marker (rearranged) chromosomes.

Patient M-5: The 5 analyzable cells from primary culture were only slightly hyperdiploid (48) and contained chromosomes with multiple rearrangements. One chromosome 1 had additional material on the long arm. There were additional copies of chromosome 6, at least 2 of them with deletions of the long arm.

Patient M-6: Nineteen of 20 cells studied from primary culture were abnormal; a single metaphase karyotyped as normal 46,XX. Among the abnormal cells, one group had counts in the hypodiploid to diploid range (43–46) and another group were hyperdiploid (80–93). The cells with lower count had a consistent 7q+ chromosome not identified in the cells with higher count. A 6p+

Table II. Growth Factor and Chromosome Studies of Nevus Cultures

Patient (nevus number)	Melanin	Monolayer Mitogen Activity	Passage Number	Number of Metaphases Karyotyped by:		Chromosome Analysis				
						Normal		Abnormal		
				Eye	Photograph	Number of Cells	Karyotype	Number of Cells	Karyotype	Percent 4n
N-1	Positive	Positive	1	29	4	6	46,XX	23	t(10;15)	1
N-2	Not studied	Negative	1	15	3	15	46,XX	0	—	0
N-3	Positive	Positive	1	41	2	41	46,XX	0	—	1
N-4	Negative	Negative[a]	1	50	7	48	46,XX	2	+8[a]	2.5
N-5[b]	Positive	Positive	1	50	5	34	46,XX	14	t(6;15)	0
								2	t(15;20)	
N-6[b]	Not studied	Negative	1	15	3	15	46,XX	0	—	1.5
N-7	Negative	Negative	1	27	3	27	46,XX	0	—	2
N-8	Negative	Positive	1–2	12	2	0	—	12	46,XX[c] t(4;5)	Not studied

[a]Subsequent analysis at passage 5–6 showed only normal chromosomes but the percentage of 4n cells rose to the abnormal value of 28%. The culture also became positive for monolayer mitogen.
[b]Two separate cultures were initiated from 2 distinct nevi from the same patient.
[c]Constitutional translocation was also present in peripheral blood.

was clearly identified in cells with higher count. Both hypo- and hyperdiploid types had a 1q+ marker, as well as many unidentifiable chromosomes ranging in number from 7 in the cells with lower count to 22 in cells with higher count. In summary, abnormalities in primary culture from M-6 included chromosome counts in 2 ranges (hypodiploid and hyperdiploid), identifiable rearrangements involving chromosomes 1, 6, and 7, and appearance of many marker chromosomes.

The 2 melanoma cell lines were both positive for growth factor assays and chromosome abnormalities (Table III). Chromosome counts were hyperdiploid and multiple rearrangements were present (not detailed here) including those involving chromosomes 1, 6, and 7.

DISCUSSION

Malignant melanoma is thought to arise not infrequently from preexisting nevi with atypical clinical and histologic features. In many instances precursor lesions are nevi which have been designated "dysplastic nevi" [1]. They may arise in 2 well-defined clinical settings: the first a dominantly inherited familial form,

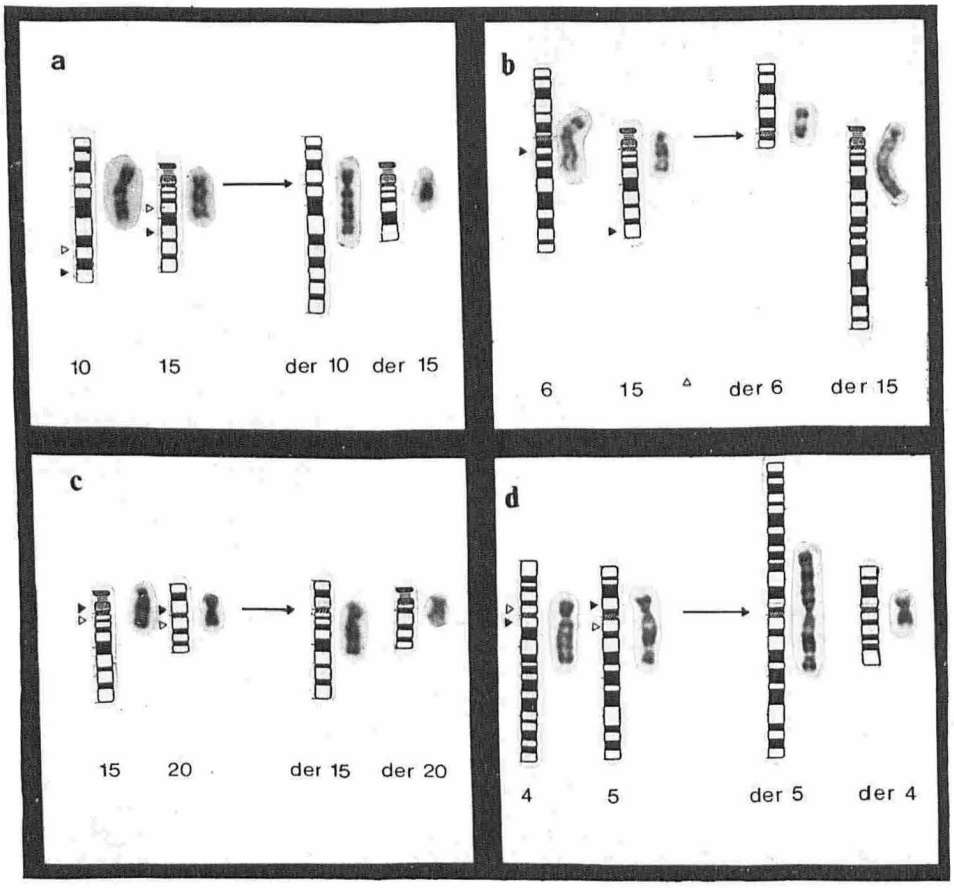

Figure 4. *a*, Models and actual photographs for the rcpt(10;15)(q26;q22) or, alternatively, rcpt(10;15)(q24;q15) in culture N-1. The first set of possible breakpoints (▶) are indicated on the normal 10 and normal 15 to the left. The alternative set of breakpoints (▷) are also indicated. Models for the derivative chromosome 10 and derivative 15 shown to the right are cut for the first set of breakpoints but models cut for the second set are similar morphologically. *b*, Models and actual photographs for the rcpt(6;15)(q13;q26) in 14 of 50 cells from culture N-5. *c*, Models and actual photographs for the rcpt(15;20)(p11.2;p11.2) or, alternatively, rcpt(15;20)(q11.2;q11.2) in 2 of 50 cells from culture N-5. The first set of possible breakpoints (▶) and alternative set (▷) are indicated. Models for the derivative 15 and 20 shown to the right are cut for the first set of breakpoints. Models cut for the second set are similar morphologically but the derived 15 would be the shorter chromosome and the derived 20 the longer. *d*, Models and actual photographs for the rcpt(4;5)(q12;p13) or alternatively rcpt(4;5)(p12;q11.2) in all cells from culture N-8. The first set of possible breakpoints (▶) and the alternative set (▷) are indicated. Models for the derivative 4 and 5 shown to the right are cut for the first set of breakpoints. Models cut for the second are similar morphologically but the derived 4 would be the longer chromosome and the derived 5 the shorter.

Table III. Growth Factor and Chromosome Studies of Melanoma Cultures

Newly Established Melanoma Cultures	Disease Stage	Melanin	Growth Factor Analysis			Chromosome Analysis						
			Monolayer Mitogen Activity	TGF[a] Activity	Passage Number	Number of Metaphases Karyotyped by:		Normal		Abnormal		Change in Chromosomes 1, 6, and/or 7
						Eye	Photograph	No. of Cells	No. of Chromosomes	No. of Cells	No. of Chromosomes	
M-1	IV	Negative	Negative	No	1	12	2	12	46,XY	0		No
M-2	III	Positive	Positive	No	Primary	42	5	40	46,XY	2	46,X,-Y	No
M-3	IV	Negative	Negative	Yes	1	11	2	7	46,XX	4	Polyploid (4n,8n)	Yes
M-4	IV	Positive	Positive	No	1	23	4	12	46,XX	11	69–75	Yes
M-5	III	Positive	Negative	Yes	Primary	5	2	0		5	48	Yes
M-6	IV	Positive	Positive	Yes	Primary	20	4	1	46,XX	6	80–93	Yes
										13	43–46	
Melanoma cell lines:												
Ca-Gor		Positive	Positive	Yes		35	17	0		35	54–67	Yes
Hs0294		Positive	Positive	Yes		49	14	0		49	60–97	Yes

[a]Activity defined as described in *Materials and Methods*.

the dysplastic nevus syndrome [12], and the second an apparently nonfamilial type referred to as the "sporadic dysplastic nevus" [13]. Kraemer et al [14] have estimated that 57% of all malignant melanomas arise from dysplastic nevi, 5.5% familial, and 52% sporadic.

Histology of the dysplastic nevus has been delineated by Clark et al [1,12]. These nevi are characterized initially by proliferation of spindle-shaped melanocytes with nuclear atypia confined to the basal cell layer of the epidermis. The next step in progression of the neoplasia occurs as a result of the continued proliferation of atypical melanocytes. This process has one of 2 recognizable patterns [1]. The first shows aggregation of distinctive atypical melanocytes into nests or theques which initially involve the entire epidermis. The second pattern shows a continuum of atypical cells 2–3 layers thick along the basal cell layer of the epidermis. There is an increase in the degree of cytologic atypia associated with the progression to malignancy consonant with development of a more malignant clone of nevocytes.

Though chromosomal abnormalities are usual in metastatic malignant melanoma, the specific time of appearance of the chromosomal abnormalities during tumor progressions is not clear. In a recent study by Balaban et al [15], 5 nevus cultures were studied, 3 of which were dysplastic, and no chromosomal abnormalities were found. In another study, flow cytometric analysis detected nuclei with abnormal (aneuploid) DNA content in only 4 of 39 congenital melanocytic nevi and in 0 of 62 acquired nevi [16]. If indeed the hyperproliferative state precedes development of chromosomal abnormalities as suggested by the first study, one would expect to find dysplastic nevi with normal karyotype but altered growth properties. Alternatively, if chromosomal abnormalities appear first, then one would expect those dysplastic nevi with altered growth control to have chromosomal abnormalities.

In this study, growth and cytogenetic abnormalities were defined in 8 nevus patients with histologically benign nevi. Four of the 8 had recognizable chromosomal abnormalities (one was constitutional). These karyotype abnormalities were accompanied by positive mitogen levels in serum-free medium conditioned by these cultures. Of the 4 remaining nevus cultures, conditioned medium from 1 had positive mitogen levels, but as yet has shown no definable karyotypic abnormalities. Histology on this nevus did, however, reveal actively proliferating theques. The other 3 nevus cultures were negative for mitogen activity and chromosome abnormality. One interpretation for these data is that in a subgroup of patients (only 1 culture in our series), nevi have undergone changes in growth control mechanisms based on growth in serum-free medium and release of mitogens into that medium, but there is no accompanying chromosomal abnormality. In another group of patients (3 cultures in our series), nevi have gone on to exhibit chromosomal and growth changes. A third category has no chromosomal or growth factor changes (3 cultures in our series). In no subgroup were there accompanying histologic abnormalities which could be identified by the pathologist as "dysplastic nevus."

The significance of the specific translocations involving chromosomes 6, 10, and 15 in the nevus cultures with chromosomal abnormalities is unclear. All of the specific breakpoints involved in the translocations are reported to be "hot spots" in human cancer and leukemia [17] except the breakpoints involved in the 6;15 translocation (patient N-5). Chromosome 15 does carry the *fes* oncogene in the region of the breakpoint of the particular translocation, however [18]. Chromosome 6 has the *myb* oncogene but not in the region of breakage for this translocation [18]. Further in vitro studies of normal or dysplastic nevi would be helpful in the delineation of the early changes in growth control and karyotype which may be involved in the progression toward melanoma in situ. It will be important to confirm whether chromosome changes, particularly reciprocal translocations, are common in nevus cultures as suggested by these studies since another study of 5 nevi (3 dysplastic) reported all karyotypes to be normal [15].

Polyploidy was not increased in the nevus cultures reported here, except patient N-4 for cultures after serial passage when 28% of cells were 4n. One step toward the hyperdiploidy of melanoma cultures could be polyploidization.

Other laboratories have reported that melanoma tumors frequently exhibit abnormalities in chromosomes 1, 6, and 7 [19–22]. Our initial cytogenetic studies of newly established melanoma cultures and 2 melanoma cell lines are in agreement with these findings but we find it difficult to assess critical, nonrandom rearrangements when many are present. It is necessary to focus on primary or low-passage melanoma cultures from early lesions in order to assess initial chromosome rearrangements. Our finding of a direct positive correlation between growth factor production and chromosome changes in melanoma cultures still does not answer the question of which comes first. Our data from one

nevus culture suggest that growth factor can be produced before observable chromosomal change. In other nevus cultures, both parameters are either absent or present.

We wish to express appreciation to Dr. John Coleman for securing the melanoma tissue on patient M-5, to Juanester Lamb for performing the mitogen assays, to Jan Fernandez, Rob Roy, Chris Engel, Susan Wilson, and Yen Wang for excellent cytogenetic assistance, and to Ronald G. Kovacs for the photomicrographs.

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